Carbon–Carbon Double Bond Formation Accompanying Hydride Transfer from a Carbanion to 5-Carbalumiflavin

Oscar L. Farng and Thomas C. Bruice*

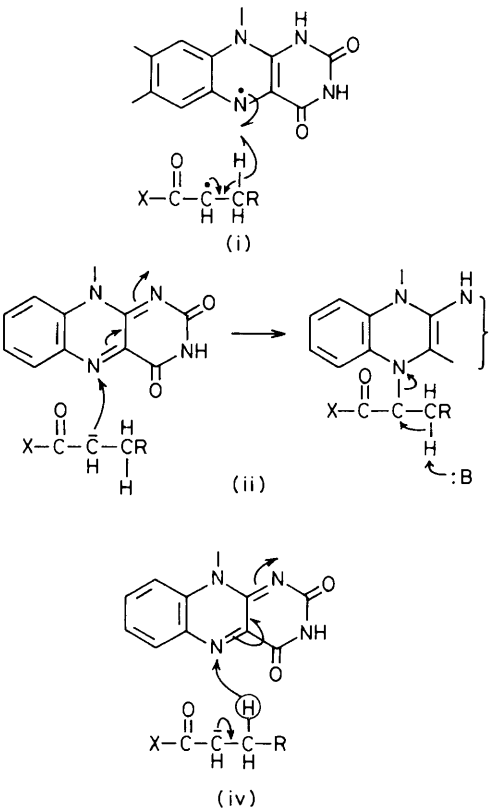
Department of Chemistry, University of California at Santa Barbara, Santa Barbara, California 93106, U.S.A.

Oxidation of the carbanions of dimethyl *trans*-(1,2-^2H_o)dihydrophthalate and the corresponding (1,2-$^2H_2$) analogue occurs by H^- and D^- transfer to the 5-position of 5-carbalumiflavin.

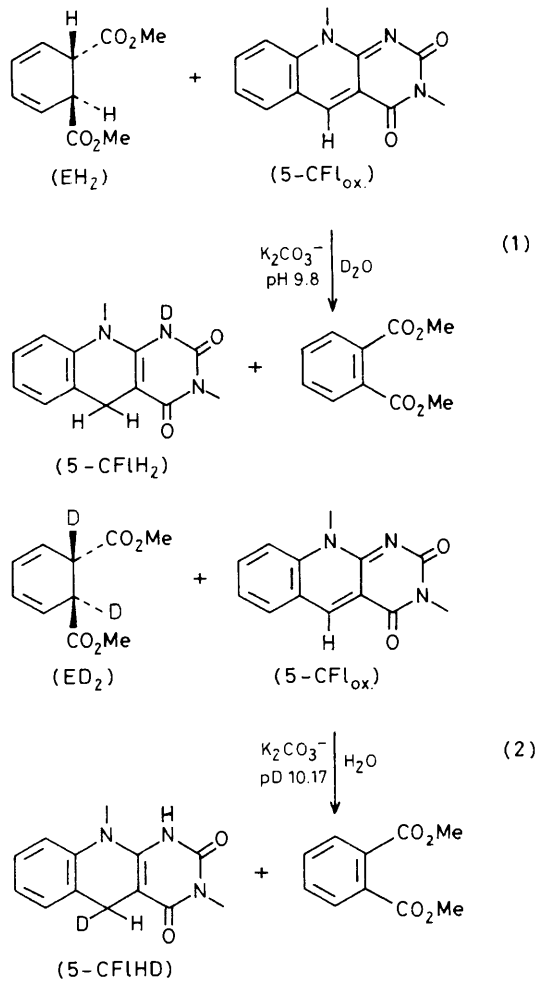
It has been shown that the flavin oxidation of reasonably acidic carbon acids in both model[1,2] and flavoenzyme[3,4] systems occurs by ionisation of the carbon acid and oxidation of the resultant carbanion. Mechanisms which have been considered for carbanion oxidation include: (i) initial $1e^-$ transfer from carbanion to flavin to provide a radical pair intermediate;[2] (ii) addition of the carbanion to the N(5)-position of the flavin to provide a covalent intermediate which yields reduced flavin and $2e^-$ oxidised substrate by general-base-catalysed fragmentation; (iii) a mechanism similar to the latter but involving nucleophilic attack at the C(4a)-position of flavin; and (iv) hydride transfer from carbanion to flavin. Hydride transfer from carbanion to flavin (iv) cannot be directly shown because of the rapid exchange of the proton on N(5) of 1,5-dihydroflavin. That H^- transfer to the N(5) position is an acceptable type of mechanism has recently been established using 1,4-dihydropyridines as the hydride source.[5] A means of determining the feasibility of hydride transfer from carbanion involves replacement of flavin by 5-carba-flavin, in which case hydrogen transfer is to a non-exchangeable position *i.e.* C(5). It was originally shown by Brustlein and Bruice[6] that NADH reduces 5-carbalumiflavin by H^- transfer to C(5) and by Shinkai and Bruice[7] that carbonyl group reduction by 1,5-dihydro-5-carbalumiflavin involves direct hydride transfer to the carbonyl carbon.

A most important type of enzymatic reaction involves the formation of α,β-unsaturation by flavoenzyme dehydrogenation of substrates possessing an ionisable proton α to a carbonyl function (*e.g.* acyl CoA dehydrogenase). In this instance oxidation of carbanion may occur by mechanisms (i), (ii), and (iv) as shown in Scheme 1. If (ii) was involved the rate-determining step would be carbanion addition to N(5) of flavin since general-base catalysis is not involved.[8,9] Herein are reported results on the oxidation of dimethyl *trans*-(1,2-^2H_o)- and dimethyl *trans*-(1,2-$^2H_2$)-dihydrophthalate (EH_2 and ED_2) by 5-carbalumiflavin (5-CFl_{ox}).

trans-(1,2-^2H_o)- and *trans*-(1,2-$^2H_2$)-dithydrophthalates were prepared from phthalic acid by Birch reduction[10] in H_2O and D_2O, respectively, and recrystallised from acetone–water. The *trans*-(1,2-^2H_o) acid has been previously reported from this laboratory[8] and the *trans*-(1,2-$^2H_2$) acid was characterised by ^1H n.m.r. spectroscopy. Esterification of both acids was accomplished with diazomethane, as reported for the protio analogue,[8] and the esters EH_2 and ED_2 were



Scheme 1



chromatographed rapidly on a short alumina column employing CCl_4 as eluant and characterised by i.r. and ^1H n.m.r. spectroscopy in CCl_4 and C_6D_6. The reaction of EH_2 with 5-$CFl_{ox.}$ was studied in D_2O whilst that of ED_2 with 5-$CFl_{ox.}$ was carried out in H_2O [reactions (1) and (2)]. In a typical run: EH_2 (2.32 mmol) in MeCN (1—2 cm^3) was added rapidly to a partial solution of 5-$CFl_{ox.}$ (0.336 mmol) in carbonate buffer (30 cm^3) at pD = 10.17. The solution was stirred for 3 days in the dark under N_2 and then filtered, and the precipitate washed with D_2O (4 cm^3). The filtrate was collected and adjusted to pD *ca.* 5 with acetic acid to precipitate 1,5-reduced 5-carbaflavin which was collected, washed with a little D_2O (pD 5), and dried under N_2. The identities of the products 5-$CFlH_2$ [reaction (1)] and 5-CFlHD [reaction (2)] were established by ^1H n.m.r. spectroscopy: δ_H (CD_3SOCD_3) 3.60 [2.1 H, s, C(5)-H_2] and 3.60 [1.1 H, br. s, C(5)-H_1], respectively.

These results establish that C–C unsaturation resulting from oxidation of the carbanion of EH_2 occurs by hydride transfer to 5-carbaflavin. This finding gains particular pertinence in view of the observations of Ghisla *et al.* that 5-carba-FMN acts as a hydride acceptor in C–C double bond formation in dehydrogenation reactions catalysed by the general acyl CoA dehydrogenase of pig kidney.[11] Care must be taken, however, in the extrapolation of results with 5-carbaflavins to oxidations by flavins, since although 5-carbaflavins have successfully replaced flavin cofactor in numerous flavoenzyme dehydrogenases, results of these studies have led to the conclusion that these compounds may more closely resemble nicotinamides than flavins.[12]

This work was supported by a grant from the National Science Foundation.

Received, 5th September 1983; Com. 1191

References

1 For reviews of the literature see: T. W. Chan and T. C. Bruice, *Biochemistry*, 1978, **17**, 4784; T. C. Bruice, *Acc. Chem. Res.*, 1980, **13**, 256.
2 M. Novak and T. C. Bruice, *J. Chem. Soc., Chem. Commun.*, 1980, 372; M. Novak and T. C. Bruice, *J. Am. Chem. Soc.*, 1977, **99**, 8079.
3 C. L. Tober, P. Nicholls and J. D. Brodie, *Arch. Biochem. Biophys.*, 1970, **138**, 506; Y. F. Cheung and C. Walsh, *Biochemistry*, 1976, **15**, 2432; C. T. Walsh, O. Lockridge, V. Massey, and R. H. Abeles, *J. Biol. Chem.*, 1973, **348**, 7049.
4 S. Ghisla and V. Massey in 'Mechanisms of Oxidizing Enzymes,' eds. T. P. Singer and R. Ondarza, Elsevier, Amsterdam, 1978, p. 55; V. Massey, S. Ghisla, and K. Kieschke, *J. Biol. Chem.*, 1980, **255**, 2796.
5 M. F. Powell and T. C. Bruice, *J. Am. Chem. Soc.*, 1983, **105**, 1014.
6 M. Brustlein and T. C. Bruice, *J. Am. Chem. Soc.*, 1972, **94**, 6548.
7 S. Shinkai and T. C. Bruice, *J. Am. Chem. Soc.*, 1973, **95**, 7526.
8 L. Main, G. J. Kasperek, and T. C. Bruice, *Biochemistry*, 1972, **11**, 3991.
9 T. C. Bruice and J. P. Taulane, *J. Am. Chem. Soc.*, 1976, **98**, 7769.
10 A. Baeyer, *Liebigs Ann. Chem.*, 1892, **269**, 189.
11 S. Ghisla, C. Thorpe, and V. Massey, manuscript in preparation.
12 C. T. Walsh, *Acc. Chem. Res.*, 1980, **13**, 148; P. Hemmel, V. Massey, and H. Fenner, *FEBS Lett.*, 1977, **84**, 5.